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(71) Applicant: Novartis AG
4058 Basel (CH)

(72) Inventors:
- Marx, Andreas
  79639 Grenzach-Wyhlen, (DE)
- Giese, Bernd, Prof.Dr.
  4051 Basel (CH)

(54) Modified nucleotides

(57) The current invention concerns new modified nucleotides that are accepted by reverse transcriptases and incorporated in to a growing oligonucleotide but are not accepted by polymerases. Oligonucleotides comprising the new modified nucleotides can be cleaved photolytically. Also embraced is a process for the synthesis of the new modified nucleotides, and their use.

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Description

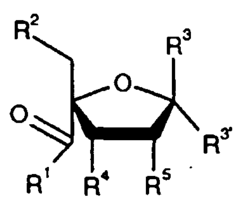
Antibiotics from the enediyne family like neocarzinostatin and esperamycin as well as metal complexes of bleomycin glycopeptides induce the oxidative cleavage of DNA by generation of highly reactive DNA radicals. In a similar way, chemical nucleases and drugs can generate oxidative stress by damage of DNA *via* radicals. The research in this area is focused on the development of new selective DNA cleavers and on the elucidation of the mechanism of action of these compounds. Whereas hydroxyl radicals react rather unselectively, several agents whose active center are bound to the minor groove preferentially abstract H-atoms from the 4'-and/or 5'-position of the deoxyribose. Recently it has been demonstrated that 4'-deoxyribosyl radicals can be generated selectively from selenides (Giese *et al., Synthesis* (1994) 1310-1312) or ketones (Giese *et al., Synlett* (1994), 1003) by photolysis. These artificial oligonucleotides could be synthesized by solid-phase synthesis with suitable substituted mononucleotides. Now, different synthetic strategies towards 4'-acyl-2'-deoxy-nucleotides with improved properties have been worked out.

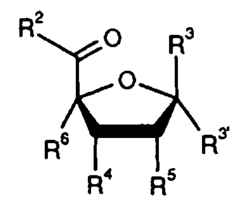
Another important feature of nucleotide analogs is their use as antiviral compounds, as for example in the treatment of AIDS or some sarcomas. Currently used nucleotide analogues like 3'-azido-2',3'-didesoxythymidine (AZT); 2',3'-didesoxy-2',3'-didehydrothymidine (d4T); 2',3'-didesoxyinosine (ddl) or 2',3'-didesoxycytidine (ddC) that are accepted by reverse transcriptases are also accepted, to a lower extent, by eukaryotic polymerases. Accordingly, when used in higher concentrations over a prolonged period the limited selectivity of these nucleotide analogues cause severe side effects.

Surprisingly, new modified nucleotides have been found that are accepted by reverse transcriptases and incorporated in to a growing oligonucleotide but not by polymerases. Usually the synthesis stops after incorporation of the inventive nucleotides. However, under certain conditions, the reverse transcriptase can be forced to continue chain elongation after incorporation of the inventive nucleotide to create an oligonucleotide that can be cleaved by light irradiation.

<u>Detailed description of the invention</u>

The current invention concerns a compound of formula 1 or 2

 (1),

 (2)

wherein

R^1	is CH_3, C_2H_5, CH_2X, CHX_2, CX_3, C(O)H, C(O)C_1-C_4alkyl, CH_2OH, CH_2-O-C_1-C_4alkyl, CH_2-O-phenyl, phenyl, or phenyl substituted with nitro or X; preferred are CH_3, C_2H_5, CF_3, CH_3OH, CH_3-O-CH_3, phenyl and phenyl substituted with nitro; more preferred are CH_3; C_2H_5 and phenyl; most preferred is CH_3;
R^2	is OR^7, mono-, di-, or triphosphate, or esters or prodrugs thereof; preferred are HO and mono-, di-, or triphosphate;
one of the residues R^3 and $R^{3'}$	is a purine or pyrimidine residue or an analogue thereof, and the other is hydrogen; preferred purine or pyrimidine radicals or an analogues thereof are adenine, inosine, N-methyladenine, N-benzoyladenine, 2-methylthioadenine, 2-aminoadenine, 6-hydroxypurine, 2-amino-6-chloropurine, 2-amino-6-methylthiopurine, guanine, N-isobutyrylguanine, uracil, thymine, cytosine, 5-fluorouracil, 5-chlorouracil, 5-bromouracil, dihydrouracil and 5-methylcytosine; and their base-protected derivatives; more preferred are adenine, inosine, guanine, uracil, thymine or cytosine; and most preferred is thymine; in a further preferred embodiment $R^{3'}$ is hydrogen;
R^4 and R^5	are independent of one another H, OR^7, O-C_1-C_4alkylNHR^7, O-C_1-C_4alkylN$R^7{}_2$,-O-(CH_2-CH_2-O)$_{1\text{-}4}R^7$ or -O-CH_2-C(OR^7)H-CH_2-OR^7; preferred are H, and OR^7; in a more preferred embodiment R^4 is OH and R^5 is H;
R^6	is H, OH, CH_2OH, CH_3, CH_2CH_3, CH_2CH_2OH; and preferably H, OH or CH_2OH;
R^7	is H or an NH or OH-protecting group; preferred is H or C_1-C_4alkyl; more preferred is H;
X	is F, Cl, Br or I; preferably F or Cl; and more preferably F;

or a pharmaceutically acceptable salt or a prodrug thereof.

In a preferred embodiment of the invention the inventive compound is of formula 1.

Purine or pyrimidine residues or analogues thereof are well known in the art as for example in WO-A-9520597 and Périgaud *et al*, Nucleosides and Nucleotides (1992), **11**, 903-945.

Protective groups and processes for derivatisation of hydroxyl groups with such protective groups are generally known in sugar and nucleotide chemistry and described, for example, by B. T. Greene, Protective Groups in Organic Synthesis, Wiley Interscience, New York (1991). Examples of such protective groups are: linear or branched C_1-C_8alkyl, particularly C_1-C_4alkyl, for example methyl, ethyl, n- and i-propyl, n-, i- and t-butyl; C_7-C_{18}aralkyl, for example benzyl, methylbenzyl, dimethylbenzyl, methoxybenzyl, dimethoxybenzyl, bromobenzyl, diphenylmethyl, di(methylphenyl)methyl, di(dimethylphenyl)methyl, di(methoxyphenyl)methyl, di(dimethoxyphenyl)methyl, trityl, tri(methylphenyl)methyl, tri(dimethylphenyl)methyl, methoxyphenyl(diphenyl)methyl, di(methoxyphenyl)phenylmethyl, tri(dimethoxyphenyl)methyl, tri(methoxyphenyl)methyl; triphenylsilyl, alkyldiphenylsilyl, dialkylphenylsilyl and trialkylsilyl having 1 to 20, preferably 1 to 12 and particularly preferably 1 to 8, C atoms in the alkyl groups, for example trimethylsilyl, triethylsilyl, tri-n-propylsilyl, i-propyldimethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, n-octyldimethylsilyl, (1,1,2,2-tetramethylethyl)dimethylsilyl; -(C_1-C_8alkyl)$_2$Si-O-Si(C_1-C_8alkyl)$_2$-, in which alkyl is, e.g., methyl, ethyl, n- or i-propyl, n-, i- or t-butyl; C_2-C_{12}acyl, particularly C_2-C_8acyl, for example acetyl, propanoyl, butanoyl, pentanoyl, hexanoyl, benzoyl, methoxybenzoyl, methylbenzoyl, chlorobenzoyl and bromobenzoyl; R^{12}-SO_2-, in which R^{12} is C_1-C_{12}alkyl, particularly C_1-C_6alkyl, C_5- or C_6cycloalkyl, phenyl, benzyl, C_1-C_{12}alkylphenyl and particularly C_1-C_4alkylphenyl, or C_1-C_{12}alkylbenzyl and particularly C_1-C_4alkylbenzyl, or halophenyl or halobenzyl, for example methyl-, ethyl-, propyl-, butyl-, phenyl-, benzyl-, p-bromo-, p-methoxy- or p-methylphenylsulfonyl; unsubstituted or F-, Cl-, Br-, C_1-C_4alkoxy-, tri(C_1-C_4alkyl)silyl- or C_1-C_4alkylsulfonyl-substituted C_1-C_{12}alkoxycarbonyl, preferably C_1-C_8alkoxycarbonyl, for example methoxy-, ethoxy-, n- or -propoxy- or n-, i- or t-butoxycarbonyl, 2-trimethylsilylethoxycarbonyl, 2-methylsulfonylethoxycarbonyl, or phenoxycarbonyl or benzyloxycarbonyl which is unsubstituted or substituted as for alkoxycarbonyl, for example methyl-or methoxy-or chlorophenoxycarbonyl or -benzyloxycarbonyl, and also 9-fluorenylmethyloxycarbonyl.

If the protecting group is alkyl, it can be substituted by F, Cl, Br, C_1-C_4alkoxy, phenoxy, chlorophenoxy, methoxyphenoxy, benzyloxy, methoxybenzyloxy or chlorophenoxy.

In a preferred embodiment, the protective groups are, independently of one another, linear or branched C_1-C_4alkyl, C_7-C_{18}aralkyl, trialkylsilyl having 1 to 12 C atoms in the alkyl groups; -(C_1-C_4alkyl)$_2$Si-O-Si(C_1-C_4alkyl)$_2$ like $(CH_3)_2$Si-O-Si$(CH_3)_2$- and -(i-C_3H_7)$_2$Si-O-Si(iC_3H_7)$_2$-; C_2-C_8acyl, R^{12}-SO_2-, in which
R^{12} is C_1-C_6alkyl; phenyl or benzyl unsubstituted or substituted with F, Cl or Br; C_1-C_4alkylphenyl; C_1-C_4alkylbenzyl; C_1-C_8alkoxycarbonyl; phenoxycarbonyl; benzyloxycarbonyl or 9-fluorenylmethoxycarbonyl.

In a particularly preferred embodiment, the protective groups are methyl, ethyl, n-or i-propyl, n-, i- or t-butyl; benzyl, methylbenzyl, dimethylbenzyl, methoxybenzyl, dimethoxybenzyl, bromobenzyl; diphenylmethyl, di(methylphenyl)methyl, di(dimethylphenyl)methyl, di(methoxyphenyl)methyl, di(methoxyphenyl)(phenyl)methyl, trityl, tri(methylphenyl)methyl, tri(dimethylphenyl)methyl, tri(methoxyphenyl)methyl, tri(dimethoxyphenyl)methyl; trimethylsilyl, triethylsilyl, tri-n-propylsilyl, i-propyldimethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, n-octyldimethylsilyl, (1,1,2,2-tetramethylethyl)dimethylsilyl, -(i-C_3H_7)$_2$Si-O-Si(i-C_3H_7)$_2$-,-$(CH_3)_2$-Si-O-Si$(CH_3)_2$-; C_1-C_8acyl groups like acetyl, propanoyl, butanoyl, pentanoyl, hexanoyl, benzoyl, methylbenzoyl, methoxybenzoyl, chlorobenzoyl and bromobenzoyl; methyl-, ethyl-, propyl-, butyl-, phenyl-, benzyl-, p-bromo-, p-methoxy- and p-methylphenylsulfonyl; methoxy-, ethoxy-, n- or i-propoxy- or n-, i- or t-butoxycarbonyl, or phenoxycarbonyl, benzyloxycarbonyl, methyl- or methoxy- or chlorophenoxycarbonyl or -benzyloxycarbonyl or 9-fluorenylmethoxycarbonyl.

Even more preferred protective groups are C_1-C_8acyl groups, for example acetyl, propanoyl, butanoyl and benzoyl.

To increase the bioavailability, the inventive compounds may be used also in form of pro-drugs, that are converted within the organism to an active agent. Usually, suitable prodrugs are modified mono- and diphosphates. These prodrugs are altered *in vivo* (by the cells) to mono- or diphosphates and than converted to triphosphates. Suitable prodrugs are described, for example, in Jones & Bischofsberger, Antiviral Res. (1995), 27, 1-17; Meier, Angew. Chem. (1996), **108**, 77-78; Nillroth *et al*., Antiviral Res. (1995), **6**, 50-64; Shimizu *et al*., Nucleosides and nucleotides (1992) **11**, 583-594; Hostetler *et al*, Proc. Natl. Acad. Sci. USA (1993), **90**, 11835-11839. Some examples are phosphonomethyl ether, S-acyl-2-thioethyl esters (SATE), dithioethyl esters, acyloxybenzyl derivates, phosphoramidates, triaryl esters, activated alkylesters, glucosyl triester, 2-nucleos-5'-O-yl-4H-1,3,2-benzodioxaphosphinin-2-oxids, dimyristoylglycerol and the like.

Also embraced by the scope of the invention are pharmaceutically acceptable salts with conventional therapeutically acceptable acids or bases. Representative inorganic acids are hydrohalic acids (such as hydrochloric acid), and also sulfuric acid, phosphoric acid and pyrophosphoric acid. Representative organic acids are in particular arene-sulfonic acids (such as benzenesulfonic or p-toluenesulfonic acid), or lower alkanesulfonic acids (such as methanesulfonic acid), as well as carboxylic acids such as acetic acid, lactic acid, palmitic acid, stearic acid, malic acid, tartaric acid, ascorbic acid and citric acid. Representative organic bases are, e.g. sodium, potassium, calcium or magnesium salts,

or also ammonium salts derived from ammonia or a pharmacologically acceptable organic nitrogen-containing base. However, if the inventive compound contains at the same time free carboxyl groups and free amino groups, it can also be obtained in the form of an inner salt.

A pharmacologically acceptable salt can be obtained from the free compound by reaction with acids, e.g. with those acids which form the above-mentioned salts, and by evaporation or lyophilization, or by adjusting the pH to a suitable neutral point, and by evaporation or lyophilization.

Another embodiment of the invention concerns the use of the inventive compound in a method of treatment. For example, one possible application is the integration of the modified nucleotides *via* the increased activity of tumor cells into the newly synthesized DNA and to kill the cells afterwards by irradiation with light at a certain wave length (by cleaving the newly synthesized DNA into pieces). Another possibility is the treatment of retro virus induced diseases like AIDS and some cancer. In a preferred embodiment of the invention the inventive compounds are used in a method of treating AIDS and retrovirus dependent cancer like, virus induced leukemia, lymphoma and sarcoma. For this purpose, the claimed active inventive compound may be administered directly or it is formed *in vivo* from a prodrug, as already explained above.

Another embodiment of the invention concerns the use of the inventive compound in a method of inhibiting the proliferation of retro viruses. Typical retrovirus are, for example, MMTV, MLV, spumavirus, HTLV, BLV, lentivirus, HIV, SIV and HSV.

The invention likewise relates to a pharmaceutical composition comprising a compound according to the invention or pharmaceutically acceptable salts thereof as active ingredients, optionally together with pharmaceutically acceptable carrier and to processes for their preparation.

The pharmaceutical preparations according to the invention which contain the compound according to the invention or pharmaceutically acceptable salts thereof are those for enteral, such as oral, furthermore rectal, and parenteral administration to a warm-blooded animal, the pharmacological active ingredient being present on its own or together with a pharmaceutically acceptable carrier. The daily dose of the active ingredient depends on the age and the individual condition and also on the manner of administration.

The pharmaceutical preparations according to the invention which contain the compound according to the invention or pharmaceutically acceptable salts thereof may be administered to a patient in various ways, e.g. parenterally, topically, enteral, such as oral, rectal or nasal. The compositions will be formulated using adjuvants and diluents suitable for the desired method of administration. Thus the compositions may be administered intravenously as bolus or by continued infusion, intramuscularly - including paravertebrally and periarticularly - subcutaneously, intracutaneously, intra-articularly, intrasynovially, intrathecally, intra-lesionally or periostally.

Parenteral compositions are preferably administered intravenously either in a bolus form or as a constant infusion. For parenteral administration, the inventive compound may be either suspended or dissolved in a sterile vehicle, optionally together with other components, and sterilized before filling into a suitable vial or ampoule and sealing. Adjuvants such as a local anesthetic, preservatives, stabilizers, solution promoters may also and/or buffers be dissolved in the vehicle. The composition may then be frozen and lyophilized to enhance stability. In the case of suspensions, a surfactant or wetting agent and/or other adjuvant as mentioned above may be included in the composition to facilitate uniform distribution of its components.

Compositions formulated for topical administration may, for example, be in aqueous jelly, oily suspension or emulsified ointment form.

Suitable carriers are, in particular, fillers, such as sugars, for example lactose, sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, furthermore binders, such as starch paste, using, for example, corn, wheat, rice or potato starch, gelatin, tragacanth, methylcellulose and/or polyvinylpyrrolidone, if desired, disintegrants, such as the abovementioned starches, furthermore carboxymethyl starch, crosslinked polyvinylpyrrolidone, agar, alginic acid or a salt thereof, such as sodium alginate; auxiliaries are primarily glidants, flow-regulators and lubricants, for example silicic acid, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol. Sugar-coated tablet cores are provided with suitable coatings which, if desired, are resistant to gastric juice, using, inter alia, concentrated sugar solutions which, if desired, contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, coating solutions in suitable organic solvents or solvent mixtures or, for the preparation of gastric juice-resistant coatings, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate. Colorants or pigments, for example to identify or to indicate different doses of active ingredient, may be added to the tablets or sugar-coated tablet coatings.

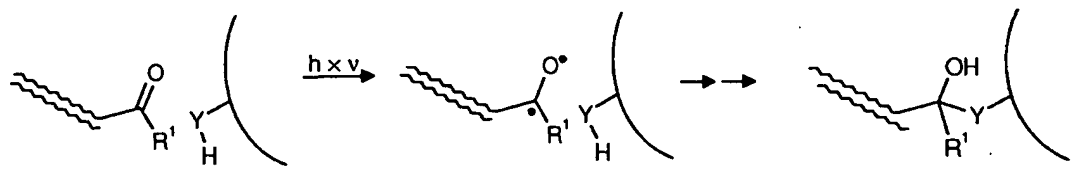
The dose of the active ingredient depends on the warm-blooded animal species, the age and the individual condition and on the manner of administration.

A further embodiment of the invention concerns the use of the inventive compound for the inhibition of reverse transcriptase like, for example, AMV RT, M-MuLV RT, HSV-RT and HIV-RT.

Another embodiment of the invention is the use of the inventive compound in a screen as for example in a screen for the presence or absence of reverse transcriptase like AMV RT, M-MuLV RT, HSV-RT and HIV-RT. In order to deter-

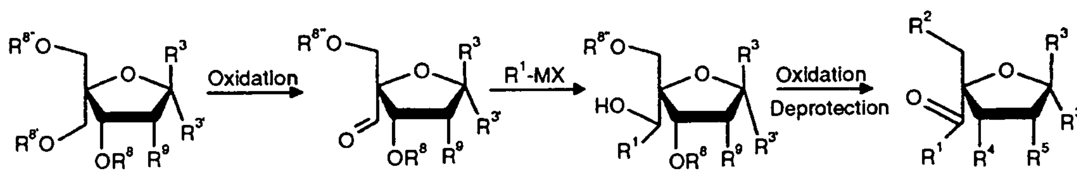
mine the amount of modified nucleotide that is inserted, the modified nucleotide may be further modified in order to ease the detection. A suitable modification in this respect is, for example, the insertion of a radioactive atom like ^{14}C, ^{32}P, ^{3}H and ^{35}S.

Another embodiment of the invention is the use of the inventive compound for the generation of photolytically cleavable DNA and RNA and analogues thereof. This can be used, for example, to generate linker with a photolytically cleavable restriction site, which are also part of the invention. This linker may be synthesized, e.g., with the aid of PCR techniques and chemically synthesized primer, comprising the inventive compound. The inventive compounds may be used also to generate single-strand gaps or to generate RNA and DNA that can be photolytically connected with a RNA or DNA binding protein as shown below.



The introduction of the inventive nucleotide into the RNA or DNA may be performed by a reverse transcriptase or by chemically synthesizing oligos that can be elongated with PCR techniques.

Also embraced by the scope of the invention is a process for the synthesis of the inventive compounds. In a preferred embodiment of the invention the process comprises the following steps:



wherein

X, R^1, R^2, R^3, $R^{3'}$, R^4 and R^5 are as defined above;
M is a metal radical that can be used in Grignard reactions like Al, Hg, Mg and Zn;
R^8, $H^{8'}$, $H^{8''}$, are. independent of one another, protecting groups; and
R^9 is hydrogen or OH^8

Suitable methods for oxidation, Grignard reactions and deprotection are well known to one skilled in the art.

Examples

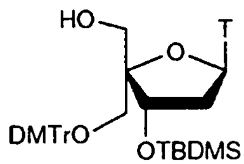
The following examples illustrate the invention and should not be construed as a limitation thereof.

Abbreviations

DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMTrCl	4,4'-dimethoxytriphenylmethyl chloride
DMTr	4,4'-dimethoxytriphenylmethyl
DMAP	4-dimethyl-aminopyridine
TBAF	tetrabutylammonium fluoride
TBDMS	tert-butyldimethylsilyl
TBDPS	tert-butyldiphenylsilyl
THF	tetrahydrofurane

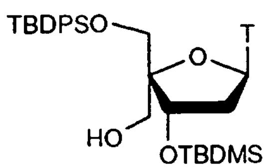
Example 1: 3'-O-tert-Butyldimethylsilyl-5'-O-tert-butyldiphenylsilyl-2'-deoxy-4'-hydroxymethyl-thymidine

2.71 g (3.93 mmol) of a compound with formula 3 (Yang *et al*, Tetrahedron Lett. (1992), **33**, 37-40; Yang *et al*, Tetrahedron Lett. (1992), **33**, 41-44).



(3)

and imidazole (0.80 g, 11.8 mmol) are dissolved in DMF (7 ml) and *tert*-butyldiphenylchlorosilane (1.11 ml, 4.33 mmol) is added at 25°C. After stirring for 24 h at 25°C the reaction mixture is poured onto water (60 ml), extracted three times with CH_2Cl_2 (80 ml each), dried over $MgSO_4$ and evaporated *in vacuo*. The resulting yellow foam is dissolved in THF (5 ml) and 80% acetic acid (17 ml) is added. After stirring at 25°C for 24 h the reaction mixture is cooled to 0°C and neutralized with a 25% aq. NH_3 soln. (20 ml), poured onto water (80 ml) and extracted twice with CH_2Cl_2 (50 ml each). The combined organic phases are washed with a sat. aq. $NaHCO_3$ soln. (100 ml), dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography (ethyl acetate/pentane 1:2) gives 1.82 g (74%) of a compound of formula 4 as a pale yellow foam.

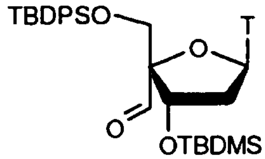


(4)

IR (NaCl): 3052, 2955, 2931, 2858, 1693, 1471, 1428, 1264, 1113, 835, 739, 703.
^{1}H-NMR ($CDCl_3$): 0.07 (*s*, CH_3—Si), 0.11 (*s*, CH_3—Si), 0.91 (*s*, *t*-Bu—Si), 1.10 (*s*, *t*-Bu—Si), 1.62 (*s*, CH_3—C(5)), 2.27 (*m*, H—C(2b'), OH), 2.36 (*ddd*, *J*=3.0, *J*=6.0, *J*=13.4, H—C(2a')), 3.63 (*dd*, *J*=8.7, *J*=12.1, H—C(5a')), 3.77 (*dd*, *J*=5.0, *J*=12.1, H—C(5b')), 3.80 (*d*, *J*=11.1, H—C(5c')), 3.88 (*d*, *J*=11.1, H—C(5d')), 4.67 (*dd*, *J*=3.0, *J*=6.4, H—C(3')), 6.41 (*dd*, *J*=6.0, *J*=7.8, H—C(1')), 7.42 (*m*, H_{arom}, H—C(6)), 7.66 (*m*, H_{arom}), 9.15 (*s*, NH).
^{13}C-NMR ($CDCl_3$): -5.2 (CH_3—Si), -5.0 (CH_3—Si), 12.0 (CH_3—C(5)), 17.9 (Me_3C—Si), 19.3 (Me_3C—Si), 25.6 ((CH_3)$_3$—C-Si), 27.0 ((CH_3)$_3$—C), 41.8(C(2')), 63.6 (C(5a')), 65.6 (C(5b')), 73.6 (C(3')), 84.2 (C(1')), 89.0 (C(4')), 111.1(C(5)), 127.9-135.5 (C_{arom}, C(6)), 150.3 (C(2)), 163.8 (C(4)).
FAB-MS: 625 (2, $[M+1]^+$). Anal. calc. for $C_{33}H_{48}N_2O_6Si_2$ (624.93):
C 63.43, H 7.74, N 4.48; found: C 63.31, H 7.83, N 4.49.

Example 2: 3'-O-tert-Butyldimethylsilyl-5'-O-tert-butyldiphenylsilyl-4'-formyl-thymidin

To a solution of trichloroacetic anhydride (0.40 ml, 2.14 mmol) in CH_2Cl_2 (6 ml) DMSO (0.20 ml, 2.98 mol) is added at -70°C. After stirring for 15 min at -70°C a solution of the compound of formula 4 (0.92 g, 1.46 mmol) in CH_2Cl_2 (2 ml) is added. After stirring for another 30 min at -70°C triethylamine (0.99 ml, 7.01 mmol) is added and the reaction mixture is warmed to 25°C within 30 min. The reaction mixture is poured onto water (60 ml), extracted three times with CH_2Cl_2 (40 ml each), dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography (ethyl acetate/pentane 1:1) gives 0.86 g (95%) of a compound of formula 5 as a pale yellow foam.



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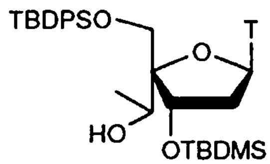
IR (NaCl): 3071, 2931, 2858, 1694, 1472, 1428, 1363, 1281, 1263, 1114, 829.
^{1}H-NMR ($CDCl_3$): 0.02 (*s*, CH_3—Si), 0.04 (*s*, CH_3—Si), 0.85 (*s*, *t*-Bu—Si), 1.10 (*s*, *t*-Bu—Si), 1.64 (*s*, CH_3—C(5)), 2.31 (*m*, H—C(2')), 3.91 (*d*, *J*=11.5, H—C(5a')), 4.11 (*d*, *J*=11.5, H—C(5b')), 4.66 (*dd*, *J*=4.8, H—C(3')), 6.70 (*dd*, *J*=8.2, H—C(1')), 7.43 (*m*, H_{arom}), 7.53 (*s*, H—C(6), 7.64 (*m*, H_{arom}), 9.09 (*s*, NH), 9.53 (*s*, C(O)H).
^{13}C-NMR ($CDCl_3$): -5.4 (CH_3—Si), -4.9 (CH_3—Si), 12.0 (CH_3—C(5)), 17.9 (Me_3C—Si), 19.3 (Me_3C—Si), 25.5 (2x$(CH_3)_3$—C-Si), 41.3(C(2')), 64.5 (C(5')), 76.2 (C(3')), 86.1(C(1')), 92.8 (C(4')), 111.5(C(5)), 127.9-135.5 (C_{arom}, C(6)), 150.3 (C(2)), 163.7 (C(4)), 200.2 (*C*HO).
FAB-MS: 623 (2, $[M+1]^+$). Anal. calc. for $C_{33}H_{46}N_2O_6Si_2$ (622.91):
C 63.63, H 7.44, N 4.50; found: C 63.28, H 7.40, N 4.39

Example 3: 3'-O-tert-Butyldimethylsilyl-5'-O-tert-butyldiphenylsilyl-2'-deoxy-4'-(1-hydroxyethyl)-thymidine

To a solution of a compound of formula 5 (0.60 g, 0.96 mmol) in THF (10 ml) a 3 M solution of CH_3MgCl (1.20 ml, 3.60 mmol) in THF is added at 0°C. After stirring for 2 h at 0°C a sat. aq. NH_4Cl soln. (20 ml) is added and extracted three times with CH_2Cl_2 (100 ml each). The combined organic phases are washed with water (100 ml), dried over $MgSO_4$ and evaporated *in vacuo* to give 0.60 g (97%) of a compound of formula 6 as a colorless foam, which is used in the next step without further purification.

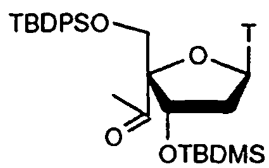


(6)

IR (KBr): 3448, 3071, 2954, 2931, 1744, 1686, 1472, 1252, 1228.
^{1}H-NMR ($CDCl_3$), diastereomer a: 0.13 (*s*, CH_3—Si), 0.17 (*s*, CH_3—Si), 0.93 (*s*, *t*-Bu—Si), 1.12 (*s*, *t*-Bu—Si), 1.17 (*d*, *J*=6.7, CH_3—CHOH), 1.54 (*d*, *J*=1.1, CH_3—C(5)), 2.35 (*m*, H—C(2')), 2.87 (*s*, OH), 4.00 (*d*, *J*=11.5, H—C(5a')), 4.10 (*d*, *J*=11.5, H—C(5b')), 4.14 (*d*, *J*=6.7, *H*—COH), 4.84 (*dd*, *J*=3.8, *J*=6.7, H—C(3')), 6.33 (*dd*, *J*=7.2, H—C(1')), 7.44 (*m*, H_{arom}, H—C(6)), 7.66 (*m*, H_{arom}), 8.52 (*s*, NH), diastereomer b: 0.11 (*s*, CH_3—Si), 0.15 (*s*, CH_3—Si), 0.93 (*s*, *t*-Bu—Si), 1.00 (*s*, *t*-Bu—Si), 1.25 (*d*, *J*=4.7, CH_3—CHOH), 1.61 (*d*, *J*=1.2, CH_3—C(5)), 2.36 (*m*, H—C(2')), 3.38 (*s*, OH), 3.60 (*d*, *J*=11.2, H—C(5a')), 3.80 (*d*, *J*=11.2, H—C(5b')), 3.84 (*d*, *J*=4.7, *H*—COH), 4.84 (*dd*, *J*=2.4, *J*=6.9, H—C(3')), 6.49 (*dd*, *J*=6.0, *J*=8.2, H—C(1')), 7.44 (*m*, H_{arom}, H— C(6)), 7.66 (*m*, H_{arom}), 8.52 (*s*, NH).
^{13}C-NMR ($CDCl_3$), diastereomer a: -5.1 (CH_3—Si), -4.3 (CH_3—Si), 12.0 (CH_3—C(5)), 17.1 (CH_3—CHOH), 17.9 (Me_3C—Si), 19.5 (Me_3C—Si), 25.7 ($(CH_3)_3$—C-Si), 27.2 ($(CH_3)_3$—C-Si), 41.6 (C(2')), 63.6 (C(5')), 68.2 (CH_3—*C*HOH), 73.6 (C(3')), 83.6 (C(1')), 89.3 (C(4')), 111.3 (C(5)), 128.0-135.5 (C_{arom}, C(6)), 150.3 (C(2)), 163.7 (C(4)), diastereomer b: - 5.1 (CH_3—Si), -4.5 (CH_3—Si), 12.1 (CH_3—C(5)), 16.8 (CH_3—CHOH), 18.8 (Me_3C—Si), 19.4 (Me_3C—Si), 27.1 ($(CH_3)_3$—C-Si), 27.2 ($(CH_3)_3$—C-Si), 42.7 (C(2')), 65.1 (CH_3—*C*HOH), 69.0 (C(5')), 74.5 (C(3')), 84.6 (C(1')), 90.8 (C(4')), 111.1(C(5)), 128.0-135.6 (C_{arom}, C(6)), 150.2 (C(2)), 163.9 (C(4)).
FAB-MS: 639 (1, $[M+1]^+$). Anal. calc. for $C_{34}H_{50}N_2O_6Si_2$(638.96):
C 63.91, H 7.89, N 4.38; found: C 63.74, H 8.04, N 4.23.

Example 4: 4'-Acetyl-3'-O-tert-butyldimethylsilyl-5'-O-tert-butyldiphenylsilyl-thymidine

1,1,1-Triacetoxy-1,1-dihydro-1,2-benzodioxol-3(1*H*)-one (0.43 g, 1.02 mmol) is dissolved in CH_2Cl_2 (7 ml) and a solution of 6 (0.32 g, 0.51 mmol) in CH_2Cl_2 (3 ml) is added at 25°C. After stirring for 1 h the reaction mixture is poured in a mixture of a sat. aq. $NaHCO_3$ soln. (10 ml) and a sat. aq. $Na_2S_2O_3$ soln. (50 ml, 1/1, v/v), extracted three times with diethylether (100 ml each), dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography (ethyl acetate/pentane 1:3) gives 0.24 g (75%) of a compound of formula 7 as a colorless foam.

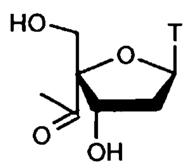


(7)

^{1}H-NMR ($CDCl_3$): 0.01 (*s*, CH_3—Si), 0.04 (*s*, CH_3—Si), 0.86 (*s*, *t*-Bu—Si), 1.10 (*s*, *t*-Bu—Si), 1.59 (*s*, CH_3—C(5)), 2.28 (*m*, H—C(2'), CH_3—C(O)), 3.95 (*d*, *J*=11.3, H—C(5')), 4.50 (*m*, H—C(3')), 6.66 (*dd*, *J*=6.3, *J*=8.7, H—C(1')), 7.42 (*m*, H_{arom}), 7.61 (*m*, H—C(6), H_{arom}), 8.98 (*s*, NH).
^{13}C-NMR ($CDCl_3$): -5.4 (CH_3—Si), -5.1 (CH_3—Si), 12.0 (CH_3—C(5)), 17.9 (Me_3C—Si), 19.5 (Me_3C—Si), 25.7 ($(CH_3)_3$—C-Si), 27.1 ($(CH_3)_3$—C-Si), 28.7 (CH_3—C(O)), 41.6 (C(2')), 66.7 (C(5')), 76.2 (C(3')), 86.3 (C(1')), 96.3 (C(4')), 111.4 (C(5)), 128.0-135.5 (C_{arom}, C(6)), 150.4 (C(2)), 163.8 (C(4)), 208.8.4 (CH_3—C(O)).
FAB-MS: 637 (3, $[M+1]^+$). Anal. calc. for $C_{34}H_{48}N_2O_6Si_2$ (636.94):
C 64.11, H 7.60, N 4.40; found: C 64.12, H 7.78, N 4.24.

Example 5: 4'-Acetyl-2'-deoxy-thymidine

To a solution of 7 (0.48 g, 0.75 mmol) in THF (15 ml) a 1M solution of TBAF (1.92 ml, 1.92 mmol) is added at 25°C and stirred for 3 h. Then, silica gel (2 g) is added to the reaction mixture and the solvent is evaporated *in vacuo*. Flash chromatography (ethyl acetate/acetonitrile 4:1) gives 0.18 g (84%) of a compound of formula 7 as a colorless foam.

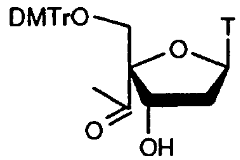


(7)

IR (KBr): 3420, 3062, 1701, 1474, 1413, 1357, 1275, 1102, 1054, 1010.
^{1}H-NMR (CD_3OD): 1.88 (*d*, *J*=1.0, CH_3—C(5)), 2.25 (*s*, CH_3—C(O)), 2.30 (*m*, H—C(2')), 3.86 (*s*, H—C(5')), 4.46 (*m*, H—C(3')), 6.58 (*dd*, *J*=5.9, *J*=9.0, H—C(1')), 7.86 (*d*, *J*=1.0, H—C(6)).
^{13}C-NMR (CD_3OD): 12.5 (CH_3—C(5)), 28.9 (CH_3—C(O)), 41.3 (C(2')), 65.5 (C(5')), 75.5 (C(3')), 87.8 (C(1')), 97.8 (C(4')), 111.8 (C(5)), 138.2 (C(6)), 152.4 (C(2)), 166.4 (C(4)), 211.7 (CH_3— C(O)).
FAB-MS: 285 (46, $[M+1]^+$). Anal. calc. for $C_{12}H_{16}N_2O_6 \cdot 0.8\ H_20$ (298.69):
C 48.21, H 5.95, N 9.48; found: C 48.18, H 5.90, N 9.12.

Example 6: 4'-Acetyl-2'-deoxy-5'-O-(4,4'-dimethoxytriphenylmethyl)-thymidine

A mixture of a compound of formula 7 (0.05 g, 0.16 mmol), DMTrCl (2 eq.) and a catalytic amount of DMAP are stirred in pyridine (4 ml/mmol) at 25°C for 24 h. After the reaction is completed, CH_3OH (1 ml/mmol) is added. The reaction mixture is poured onto a sat. aq. $NaHCO_3$ soln. (100 ml/mmol) and extracted three times with CH_2Cl_2 (50 ml each), dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography (ethyl acetate/pentane/triethylamine 1:1:0.01) gives 0.07 g (72%) of a compound of formula 8 as a pale yellow foam.

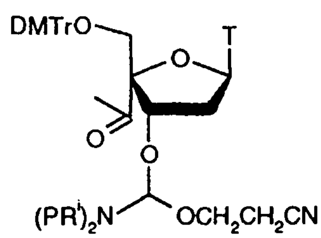


(8)

IR (KBr): 3447, 3064, 2955, 1688, 1607, 1509, 1297, 1177, 1092, 1034, 830.
^{1}H-NMR ($CDCl_3$): 1.42 (*s*, CH_3—C(5)), 2.30 (CH_3—C(O)), 2.43 (*m*, H—C(2')), 3.12 (s, HO—C(3')), 3.42 (*d*, *J*=9.9, H—C(5a')), 3.55 (*d*, *J*=9.9, H—C(5b')), 3.79 (*s*, CH_3O), 4.65 (*d*, *J*=3.8, H—C(3')), 6.68 (*dd*, *J*=5.7, *J*=8.9, H—C(1')), 6.84 (*m*, H_{arom}), 7.30 (*m*, H_{arom}), 7.56 (*s*, H—C(6)), 9.20 (*s*, NH).
^{13}C-NMR ($CDCl_3$): 11.7 (CH_3—C(5)), 28.7 ($\underline{C}H_3$—C(O)), 40.1 (C(2')), 55.3 (CH_3—O), 66.4 (C(5')), 75.0 (C(3')), 86.0 (C(1')), 87.6 ($\underline{C}Ar_3$), 95.7 (C(4')), 111.7 (C(5)), 113.4 (C_{arom}—*ortho* to OMe), 127.3-144.0 ($C_{arom.}$ C(6)), 150.5 (C(2)), 158.9 (C_{ipso}—OMe), 163.8 (C(4)), 211.6 (CH_3— *C*(O)).
FAB-MS: 587 (2, $[M+1]^+$). Anal. calc. for $C_{33}H_{34}N_2O_8$ (586.65):
C 67.56, H 5.84, N 4.78; found: C 67.22, H 6.37, N 4.29.

Example 7: O-{3-[4-acetyl-2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-1-(1-thymyl)]-β-D-erythro-pento-1,4-furanosyl}-O-(2-cyanoethyl)-N,N'-diisopropyl-phosphoramidite

The compound of formula 8 (0.06 g, 0.09 mmol), *N,N*-diisopropylethylamine (5.5 eq) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (2.3 eq) are dissolved in CH_2Cl_2 (4 ml / mmol) and stirred for 2 h at 25°C. The reaction mixture is diluted with CH_2Cl_2 (20 ml) and hydrolyzed with a sat. aq. $NaHCO_3$ soln. (100 m/mmol), extracted twice with CH_2Cl_2 (50 ml each), dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography (ethyl acetate/pentane/triethylamine 2:1:0.01) gives 0.06 g (78%) of the 3'-O-phosphoramidite of formula 9 as a pale yellow foam.

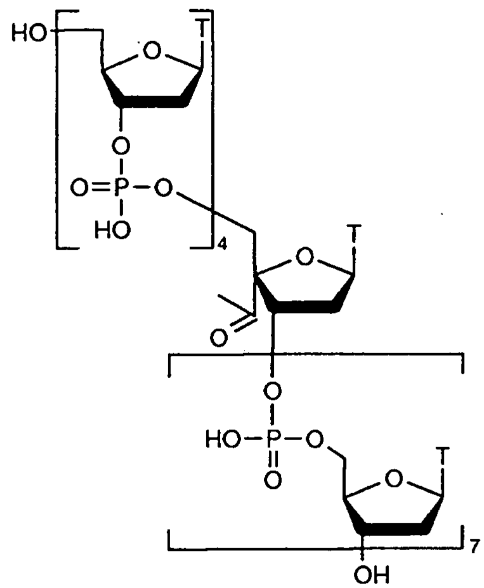


(9)

IR (KBr): 3447, 3059, 2967, 2931, 1700, 1654, 1509, 1509, 1466, 1252, 1179, 1032, 831.
^{1}H-NMR ($CDCl_3$), mixture of diastereomers: 1.25 (*m*, $CH(CH_3)_2$, CH_3—C(5)), 2.45 (*m*, H-C(2'), CH_2CN, CH_3—C(O)), 3.60 (*m*, CH_2—OP, CH—N, H—C(5'), CH_3O), 4.76 (m, H—C(3')), 6.66 (*m*, H—C(1')), 6.85 (*m*, H_{arom}), 7.31 (*m*, H_{arom}), 7.58 and 7.60 (*d*, *J*=1.0, H—C(6)).
^{13}C-NMR ($CDCl_3$), mixture of diastereomers: 11.7 (CH_3—C(5)), 20.3-20.5 (CH_2CN), 24.3-24.7 ($N(CH(CH_3)_2)$), 28.6 (CH_3—C(O)), 39.7 ($N(CH(CH_3)_2)$), 43.4 and 43.62 (2*d*, J_a=12.4, J_b=12.5, C(2')), 55.3 (CH_3—O), 58.0 and 58.66 (2*d*, J_a=20.4, J_b=19.0, CH_2—OP), 65.9 and 66.0 (C(5')), 76.8 (C(3')), 86.0 and 86.1 (C(1')), 87.6 (CAr_3), 94.4 (*d*, *J*=5.8, C(4')), 111.5 (C(5)), 113.4 (C_{arom}—*ortho* to OMe), 117.5 (CN), 127.3-144.0 (C_{arom}, (C(6)), 150.3 (C(2)), 158.9 (C_{ipso}— OMe), 163.8 (C(4)), 208.0 and 208.2 (CH_3—*C*(O)).
^{31}P-NMR ($CDCl_3$), diastereomer a: 150.1, diastereomer b: 150.0.
FAB-MS: 787 (2, $[M+1]^+$). Anal. calc. for $C_{42}H_{51}N_4O_9P \cdot 0.5\ H_2O$ (795.87):
C 63.32, H 6.52, N 7.00; found: C 62.97, H 6.50, N 6.85.

Example 8: Solid-phase synthesis of the 4'-modified oligonucleotides (5'-d($T_4T^*T_7$))

The synthesis of oligonucleotides of formula 10

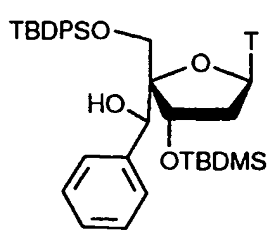


(10)

is carried out on an *ABI 392 DNA/RNA* synthesizer in a 1 μmol scale (20 mol equiv. phosphoramidite per cycle, 500 Å CPG support). The standard procedure for β-cyanoethylphosphoramidites is used, except that the coupling time of the modified nucleoside e3 is extended to 30 min. The coupling efficiencies of the modified building blocks e3 are similar to those of the commercially available amidites (98%, assigned by conductivity measurements of the trityl salt released on each cycle). Concentrated NH_3 is used to remove the oligonucleotides from the solid support (55°C, 8 h). The crude oligonucleotides are detritylated and desalted on oligonucleotide cartridges (OPC, *MWG-Biotech*). Preparative HPLC (RP-18, linear gradient of 5-40% acetonitrile (20 min) in 0.1% triethylammonium acetate of pH = 7.0) leads after lyophilyzation to the oligonucleotides 10. MALDI-TOF MS: 3629.2 (calc. 3929.6) $(M-H^+)^-$ for 10.

Example 9: 3'-O-tert-Butyldimethylsilyl-5'-O-tert-butyldiphenylsilyl-2'-deoxy-4'-(1-hydroxybenzyl)-thymidine

CuI (0.04 g, 0.20 mmol) is suspended in a solution of a compound of formula 5 (0.70 g, 1.12 mmol) in THF (50 ml) at -5°C and a 1 M solution of phenylmagnesium bromide (6.00 ml, 6.00 mmol) is added slowly. The reaction mixture is stirred for 2 h at - 5°C and for 1 h at 0°C. Then, a sat. aq. NH_4Cl soln. (20 ml) is added, the water phase is extracted seven times with diethylether (50 ml each). The organic phase is dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography (ethyl acetate/pentane 1:2) yields 0.62g (79%) of a compound of formula 11 as a colorless foam.



(11)

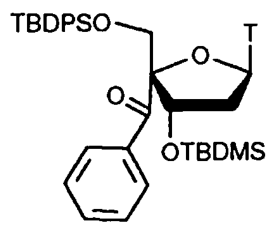
^{1}H-NMR ($CDCl_3$): 0.17 (*s*, CH_3—Si), 0.18 (*s*, CH_3—Si), 0.97 (*s*, *t*-Bu—Si), 1.07 (*s*, *t*-Bu—Si), 1.57 (*d*, *J*=1.2, CH_3—C(5)), 2.15 (*m*, H—C(2')), 2.34 (*m*, H—C(2')), 3.26 (*d*, *J*=11.1, H—C(5')), 3.46 (*d*, *J*=11.1, H—C(5')), 4.04 (*d*, *J*=2.7, OH), 4.82 (*d*, *J*=2.9, H—C(3')), 4.94 (*dd*, *J*=7.5, *J*=2.1, H-C(5')), 6.59 (*dd*, *J*=7.8, *J*=6.3, H—C(1')), 7.40 (*m*, C_6H_5 and H—C(6)), 8.6 (*s*, NH).

^{13}C-NMR ($CDCl_3$): -4.7 (CH_3—Si), -4.2 (CH_3—Si), 12.3 (CH_3—C(5)), 18.2 ((CH_3)$_3$*C*—Si) 19.9 ((CH_3)$_3$*C*—Si), 26.0 ((*C*H$_3$)$_3$—C-Si), 27.4 ((CH_3)$_3$—C-Si), 42.5 (C(2')), 66.4 (C(5')), 75.6 (C(5')) 75.9 (C(3')), 85.5 (C(1')), 90.5 (C(4')), 111.3 (C(5)), 128.2-139.0 (C(6), C_{arom}), 150.2 (C(2)), 163.6 (C(4)).

FAB-MS: 701 (1.5, $[M+1]^+$). Anal. calc. for $C_{39}H_{52}N_2O_5Si_2 \cdot 0.25\ H_2O$ (703.51):
C 66.82, H 7.48, N 4.00; found: C 66.82, H 7.47, N 3.84.

Example 10: 4'-Benzoyl-3'-O-tert-butyldimethylsilyl-5'-O-tert-butyldiphenylsilyl-2'-deoxythymidine

1,1,1-Triacetoxy-1,1-dihydro-1,2-benzodioxol-3(1*H*)-one (0.43 g, 1.02 mmol) is dissolved in CH_2Cl_2 (10 ml) and a solution of a compound of formula 11 (0.46 g, 0.66 mmol) in CH_2Cl_2 (5 ml) is added at 25°C. After stirring for 1 h the reaction mixture is poured in a mixture of a sat. aq. $NaHCO_3$ soln. (10 ml) and a sat. aq. $Na_2S_2O_3$ soln (60 ml, 1/1, v/v), extracted three times with diethylether (100 ml each), dried over $MgSO_4$ and evaporated *in vacuo* to give 0.45 g (99%) of a compound of formula 12



(12)

as a colorless foam which is used in the next step without further purification.

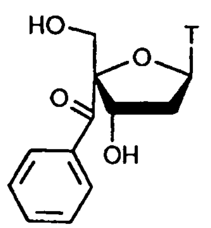
^{1}H-NMR ($CDCl_3$): -0.06 (*s*, CH_3—Si), 0.06 (*s*, CH_3—Si), 0.74 (*s*, *t*-Bu—Si), 1.61 (*d*, J=1.2, CH_3— C(5)), 2.33 (*m*, H—C(2')), 2.40 (*m*, H—C(2')), 4.21 (*d*, *J*=11.1, H—C(5')), 4.22 (*d*, *J*=11.1, H— C(5')), 4.64 (*d*, *J*=4.8, H—C(3')), 6.64 (*dd*, *J*=9.3, *J*=6.3, H—C(1')), 7.59 (*m*, H—C(6), H_{arom}), 8.16 (*s*, NH).

^{13}C-NMR ($CDCl_3$): -5.2 (CH_3—Si), -4.9 (CH_3—Si), 12.0 (*C*H$_3$—C(5)), 17.9 (Me$_3$*C*—Si), 19.4 (Me$_3$*C*—Si), 25.6 ((CH_3)$_3$—C-Si), 27.0 ((*C*H$_3$)$_3$—C-Si), 41.7 (C(2')), 68.4 (C(5')), 77.2 (C(3')), 86.5 (C(1')), 98.5 (C(4')), 110.9 (C(5)), 127.9-135.6 (C(6), C_{arom}), 150.0 (C(2)), 163.4 (C(4)), 201.4 (C(5')).

FAB-MS: 699 (2.6, $[M+1]^+$). Anal. calc. for $C_{39}H_{50}N_2O_6Si$ (698.32):
C 66.50, H 7.17, N 3.98; found: C 66.45, H 7.11, N 4.04.

Example 11: 4'-Benzoyl-2'-deoxy-thymidine

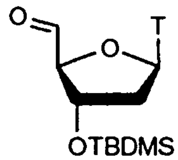
To a solution of a compound of formula 12 (6.50 mg, 0.01 mmol) in THF (2 ml) a 1 M solution of TBAF (0.01 ml, 0.01 mmol) is added at 0°C. After stirring for 1 h at 0°C the reaction mixture is directly chromatographed (dichloromethane/methanol 16:1) to remove TBAF. After evaporation *in vacuo*, the residue is dissolved in THF (2 ml) and pyridinium polyhydrogen fluoride (0.07 ml, 70% HF) is added at 25°C. After stirring for 2 d the reaction mixture is poured onto a sat. aq. $NaHCO_3$ soln. (25 ml), extracted four times with ethyl acetate (50 ml each), dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography (dichloromethane/methanol 16:1) gives 0.60 mg (18%) of a compound of formula 13.



(13)

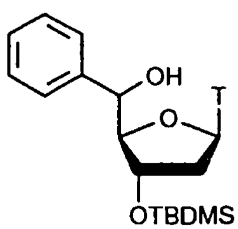
Example 12: 3'-O-tert-Butyldimethylsilyl-2'-deoxy-5'-C-phenyl-thymidine

$CeCl_3 \cdot 7\,H_2O$ (0.63 g, 1.70 mmol) is placed in a 100 ml three-necked flask and heated under stirring at 140°C *in vacuo* (0.1 Torr) for 10 h. After cooling to 0°C, THF (10 ml) is added and stirring is continued for 15 h. The mixture is then agitated at - 5°C for 10 min and a solution of aldehyde of formula 14 (Yang *et al.*, Tetrahedron Lett. (1992), **33**, 41)



(14)

(0.20 g, 0.56 mmol) in THF (10 ml) is added. Stirring at -5°C is continued for 15 min. Phenylmagnesium bromide (1.7 ml of a 1 M solution in THF, 1.70 mmol) is added slowly to the resulting suspension whereupon the color of the solution changes from white to yellow. After 2 h at -5°C and 1 h at 10°C, the mixture is quenched with a sat. aq. NH_4Cl soln. (25 ml), the resulting pale yellow suspension is poured onto water (100 ml) and extracted three times with diethylether (100 ml each), dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography (dichloromethane/ethanol 20:1) gives 0.20 g (82%) of a diastereomeric mixture of the compound of formula 15 as a colorless foam.



(15)

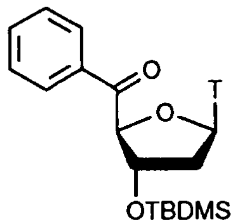
An alternative procedure to synthesize the compound of formula 15 is:
The aldehyde of formula 4 (2.00 g, 5.60 mmol) is dissolved in THF (80 ml) and cooled to -5°C. Cu (0.21 g, 1.10 mmol) is added to the resulting colorless solution, the mixture is agitated for 10 min and phenylmagnesium bromide (3.40 ml of a 1 M solution in THF, 3.40 mmol) is added. After stirring for 2 hours at -5°C the reaction mixture is hydrolyzed with a sat. NH_4Cl soln. (100 ml) and extracted eight times with diethylether (100 ml each), dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography (methylene chloride/ethanol 20:1) gives 1.90 g (78%) of a diastereomeric mixture of the compound of formula 15 as a colorless foam.

^{1}H-NMH ($CDCl_3$), diastereomer a: 0.02 (*s*, CH_3—Si), 0.86 (*s*, CH_3—Si), 1.92 (*s*, *t*-Bu—Si), 1.92 (*s*, CH_3—C_5), 2.13-2.21 (*ddd*, *J*=2.8, *J*=5.5, *J*=10.3, H—C(2b')), 2.30-2.39 (*m*, H—C(2a')), 3.43 (*d*, *J*=4.9, HO—C(5')), 4.08 (*dd*, *J*=3.3, *J*=3.8 H—C(4')), 4.52 (*m*, H—C(3')), 4.81 (*dd*, *J*=3.8, J=4.9, H—C(5')), 6.11 (*dd*, *J*=5.5, *J*=8.0, H—C(1')), 7.28-7.52 (*m*, H_{arom}, H—C(6)), 9.24 (s, NH)) diastereomer b: 0.09 (*s*, CH_3—Si), 0.10 (*s*, CH_3—Si), 0.90 (*s*, *t*-Bu—Si), 1.01 (*s*, *t*-Bu—C), 1.93 (*d*, *J*=1.2, CH_3—C(5)), 2.05-2.22 (*m*, H—C(2b')), 2.36-2.38 (*m*, H—C(2a')), 2.82-2.83 (*m*, HO— C(5')), 3.50 (*m*, H—C(5')), 4.07 (*t*, *J*=2.2, H—C(4')), 4.61 (*dt*, *J*=5.7, *J*=2.1, H—C(3')), 6.09 (*dd*, *J*=8.7, *J*=5.8, H—C(1')), 7.28-7.52 (*m*, H_{arom}, H—C(6)), 8.22 (s, NH).
^{13}C-NMR ($CDCl_3$), diastereomer a: -4.7 (CH_3—Si), -4.6 (CH_3—Si), 12.5 (CH_3—C(5)), 18.0 (Me_3C—Si), 26.5 ((CH_3)$_3$—C), 39.7 (C(2')), 74.3 (C(5')), 78.3 (C(3')), 86.2 (C(1')), 87.1 (C(4')), 111.0 (C(5)), 125.9-128.8 (C_{arom}), 137.4 (C(6)), 150.6 (C(2)), 164.1 (C(4)), diastereomer b: - 4.9 (CH_3—Si), -4.0 (CH_3—Si), 12.4 (CH_3—C(5)), 17.9 (Me_3C—Si), 25.7 ((CH_3)$_3$—C-Si), 40.7 (C(2')), 72.4 (C(5')), 80.5 (C(3')), 86.0 (C(1')), 88.7 (C(4')), 110.9 (C(5)), 125.9-128.8 (C_{arom}), 136.9 (C(6)), 150.4 (C(2)), 163.95 (C(4)).
FAB-MS: 433 (11, $[M+1]^+$). Anal. calc. for $C_{22}H_{32}N_2O_5Si$ (432.60):
C 61.08, H 7.46, N 6.48; found: C 60.76, H 7.70, N 6.48.

Example 13: 1-(3-O-tert-butyldimethylsilyl-2-deoxy-5-oxo-5-C-phenyl-β-D-erythro-pento-1,4-furanosyl)-thymine

To a solution of trifluoroacetic anhydride (0.30 ml, 2.23 mmol) in CH_2Cl_2 (5 ml), DMSO (0.30 ml, 4.14 mmol) in CH_2Cl_2 (4 ml) is added dropwise at -60°C. The mixture is stirred for 30 min at -60°C. Then, a solution of compound of

formula 15 (0.27 g, 0.62 mmol) in CH_2Cl_2 (10 ml) is added slowly. Agitation for one hour at -60°C is followed by the addition of NEt_3 (0.5 ml, 165 mmol). The reaction mixture is diluted with CH_2Cl_2 (20 ml) hydrolyzed with a sat. aq. $NaHCO_3$ soln. (50 ml), extracted four times with CH_2Cl_2 (75 ml each), dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography (pentane/ether/dichloromethane 3:3:4) yields 0.23 g (85%) of a compound of formula 16 as a colorless foam.

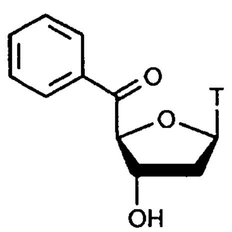


(16)

IR (KBr): 3325, 3185, 3065, 2954, 2923, 2855, 1695, 1471, 1278, 1218, 1133, 1085, 982, 837, 777, 692.
^{1}H-NMR ($CDCl_3$): 0.07 (*s*, CH_3—Si), 0.08 (*s*, CH_3—Si), 0.95 (*s*, *t*-Bu—Si), 2.02 (*s*, CH_3—C(5)), 2.01 (*ddd*, *J*=3.5, *J*=5.5, *J*=11.6, H-C(2b')), 2.35 (*dd*, *J*=5.4, *J*=11.6, H—C(2a')), 4.57 (*d*, *J*=5.4, H—C(3')), 5.45 (*d*, *J*=1.4, H—C(4')), 6.59 (*dd*, *J*=5.4, *J*=8.8, H—C(1')), 7.55 (*t*, *J*=7.7, *m*-H_{arom}), 7.68 (*t*, *J*=7.1, *p*-H_{arom}), 8.02 (*d*, *J*=7.2, *o*-H_{arom}), 8.23 (s, H—C(6)).
^{13}C-NMR ($CDCl_3$): -4.99 (CH_3—Si), -4.64 (CH_3—Si), 12.69 (CH_3—C(5)), 17.83 (Me_3C—Si), 25.60 ($(CH_3)_3$—C-Si), 39.99 (C(2')), 74.63 (C(3')), 86.12 (C(1')), 87.60 (C(4')), 111.2 (C(5)), 128.5-134.5 (C_{arom}), 136.2 (C(6)), 150.4 (C(2)), 163.8 (C(4)), 197.0 (PhCO). FAB-MS: 431 (31, $[M+1]^+$). Anal. calc. for $C_{22}H_{30}N_2O_5Si$ (430.57): C 61.37, H 7.02, N 6.51; found: C 61.05, H 7.12, N 6.36.

Example 14: 1-(2-deoxy-5-C-phenyl-5-oxo-β-D-erythro-pento-1,4-furanosyl)-thymine

A solution of the compound of formula 16 (0.15 g, 0.36 mmol) in THF (10 ml) is treated with a 1 M solution of TBAF (0.40 ml, 0.40 mmol) in THF at 0°C. After stirring for 45 min at 0°C the reaction mixture is directly chromatographed without previous work up. Flash chromatography (dichloromethane/methanol 20:1) gives 0.10 g (89%) of a compound of formula 17 as a colorless foam.

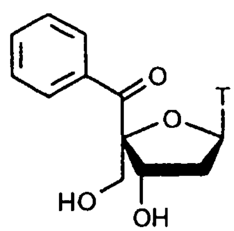


(17)

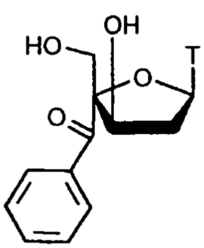
IR (KBr): 3325, 3185, 3065, 2954, 2923, 2855, 1695, 1471, 1278, 1218, 1133, 1085, 982, 837, 777, 692.
^{1}H-NMR ($CDCl_3$+10% CD_3OD): 1.93 (*ddd*, *J*=2.8, *J*=5.0, *J*=13.6, H—C(2b')), 2.01 (*s*, CH_3— C(5)), 2.42 (*dd*, *J*=5.2, *J*=13.6, H—C(2a')), 4.59 (*d*, *J*=5.0, H—C(3')), 5.52 (*d*, *J*=1.1, H—C(4')), 6.58 (*dd*, *J*=5.0, *J*=9.3, H—C(1')), 7.55 (*dd*, *J*=7.3, *J*=7.6, *m*-H_{arom}), 7.67 (*t*, *J*=7.4, *p*-H_{arom}), 8.07 (*d*, *J*=7.2, *o*-H_{arom}), 8.37 (s, H—C(6)).
^{13}C-NMR ($CDCl_3$ +10% CD_3OD): 12.27 (CH_3—C(5)), 39.04 (C(2')), 73.47 (C(3')), 86.02 (C(1')), 87.45 (C(4')), 111.0 (C(5)), 128.1-134.3 (C_{arom}), 136.6 (C(6)), 150.1 (C(2)), 164.5 (C(4)), 197.1 (PhCO).
FAB-MS: 317 (15, $[M+1]^+$). Anal. calc. for $C_{16}H_{16}N_2O_5$ (316.32):
C 60.75, H 5.10, N 8.86; found: C 61.19, H 5.48, N 8.55.

Example 15: 4'-C-Benzoyl-2'-deoxy-thymidine, 1-(4-benzoyl-2-deoxy-α-L-threo-pento-1,4-furanosyl)-thymine, and 1-(4-benzoyl-2-deoxy-β-D-threo-pento-1,4-furanosyl)-thymine

$Ba(OH)_2 \cdot 8\ H_2O$ (0.12g, 0.68 mmol) is suspended in a solution of a compound of formula 17 (0.18 g, 0.56 mmol) in dioxane/water 10:1 (3.6 ml). After addition of a 36% aq. form-aldehyde soln. (0.30 ml, 3.60 mmol), the mixture is sonicated for 30 sec (sonication bath). Stirring at 25°C is continued for 1 h. Then, the reaction mixture is frozen with liquid N_2 and lyophylized *in vacuo*. Flash chromatography of the white residue (dichloromethane/methanol 20:1) gives 38 mg (22 %) of a compound of formula 19 and 126 mg (73%) of the mixture of compounds of formulae 18:13 (2.5:1) as colorless crystals.



(18)



(19)

Prep. HPLC (*Knauer*, RP-18, 7μm; length 250mm, diameter 15mm; flow: 6ml/min; H_2O/CH_3CN = 81:19) gives 38 mg compound of formula 13 (22%) and 72 mg compound of formula 18 (42%) as colorless crystals.

Data for compound of formula 13:

IR (KBr): 3442, 3065, 2935, 1692, 1633, 1512, 1141, 1042, 953, 752.
^{1}H-NMR ($CDCl_3$ + 10% CD_3OD):1.92 (*s*, CH_3—C(5)), 2.39 (*ddd*, *J*=1.5, *J*=5.6, *J*=9.0, H-C(2b')), 2.47 (*ddd*, *J*=4.0, *J*=5.2, *J*=9.0, H—C(2a')), 3.96 (*d*, *J*=11.6, H—C(5b')), 4.06 (*d*, *J*=11.6, H—C(5a')), 4.94 (*m*, H—C(3')), 6.46 (*dd*, *J*=4.0, *J*=5.6, H—C(1')), 7.45 (*t*, *J*=7.9, *m*-H_{arom}), 7.51 (*t*, *J*=6.7, *p*-H_{arom}), 7.82 (*s*, H—C(6)), 7.92 (*d*, *J*=7.2, *o*-H_{arom}). NOE (^{1}H-NMR DMSO-d_6): (irradiated H → affected H; ++ = strong, + = medium, (+) = weak): H—C(6)→H—C(5')(+) and H—C(1')(++); *o*-H_{arom}→H—C(5')(+) and H—C(1')((+)); H—C(1')→ *o*-H_{arom}(+) and H—C(6)(+); H—C(3')→H-C(5')(+); H—C(5')→*o*-H-Ph(+) and H—C(6)(+) and H—C(3'); H—(C2'a)→H—C(6)(+).
^{13}C-NMR ($CDCl_3$ + 10% CD_3OD): 12.35 (CH_3—C(5)), 40.07 (C(2')), 65.31 (C(5')), 74.19 (C(3')), 85.12 (C(1')), 97.67 (C(4')), 109.6 (C(5)), 127.8-136.1 (C_{arom}), 138.3 (C(6)), 150.5 (C(2)), 163.7 (C(4)), 202.3 (Ph*C*O).
FAB-MS: 347 (33, $[M+1]^+$). Anal. calc. for $C_{17}H_{18}N_2O_6 \cdot 0.75\ H_2O$ (359.85):
C 56.67, H 5.60, N 7.78; found: C 56.61, H 5.54, N 7.74.

Data for compound of formula 18:

IR (KBr): 3454, 3041, 2899, 1701, 1687, 1537, 1049, 953, 752, 672.
^{1}H-NMR (DMSO-d_6): 1.36 (*s*, CH_3—C(5)), 2.03 (*m*, H—C(2b'), H—C(2a')), 3.84 (*dd*, *J*=3.3, *J*=11.6, H—C(5b')), 4.00 (*dd*, *J*=4.9, *J*=11.6, H—C(5a')), 4.75 (*m*, H—C(3')), 5.13 (*m*, OH), 5.75 (*m*, -OH), 6.39 (*dd*, *J*=6.2, *J*=8.5, H—C(1')), 6.59 (*s*, H—C(6)), 7.44 (*t*, *J*=7.9, *m*-H_{arom}), 7.56 (*t*, *J*=7.3, *p*-H_{arom}), 7.92 (*d*, *J*=7.1, *o*-H_{arom}), 11.2 (s, -NH-).
NOE (^{1}H-NMR DMSO-d_6): (irradiated H → affected H; ++ = strong, + = medium, (+) = weak): *o*-H_{arom}→H—C(6)(+); H—C(6)→H—C(3')(+) and *o*-H_{arom}((+)) and H—C(1')(++); H—C(1')→H-C(5')((+)); H—C(3')→H-C(6)((+)); H-C(5')→*o*-$H_{arom.}$ (+) and H—C(1')((+)) and HO—C(3')(+).
^{13}C-NMR (DMSO-d_6):12.07 (CH_3—C(5)), 38.22 (C(2')), 64.65 (C(5')), 74.45 (C(3')), 84.92 (C(1')), 97.00 (C(4')),

108.9 (C(5)), 127.7-135.7 (C_{arom}), 138.8 (C(6)), 150.5 (C(2)), 163.4 (C(4)), 203.7 (Ph*C*O).
FAB-MS: 347 (32, $[M+1]^+$). Anal. calc. for $C_{17}H_{18}N_2O_6 \cdot 0.75\ H_2O$ (359.85):
C 56.67, H 5.60, N 7.78; found: C 56.55, H 5.68, N 7.71.

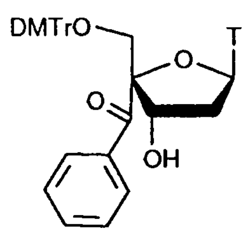
Data for compound of formula 19:

IR (KBr): 3418, 3066, 2929, 1685, 1596, 1279, 1104, 1052, 953, 786, 693.
^{1}H-NMR ($CDCl_3$ + 10% CD_3OD):1.95 (*s*, CH_3—C(5)), 2.06 (*ddd*, *J*=2.6, *J*=3.9, *J*=14.8, H— C(2b')), 2.49 (*dd*, *J*=5.8, *J*=14.8, H—C(2a')), 4.21 (*d*, *J*=11.8, H—C(5b')), 4.29 (*d*, *J*=11.8, H—C(5a')), 4.94 (*m*, H—C(3')), 6.07 (*dd*, *J*=3.9, *J*=8.0, H—C(1')), 7.45 (*dd*, *J*=7.7, *J*=8.0, *m*-H_{arom}), 7.56 (*t*, *J*=7.5, *p*-H_{arom}), 7.95 (*s*, H—C(6)), 8.12 (*d*, *J*=7.2, *o*-H_{arom}).
NOE (^{1}H-NMR DMSO-d_6): (irradiated H→affected H; ++ = strong, + = medium, (+) = weak): *o*-H_{arom}→H—C(5')(+) and H—C(1')((+)); H—C(6)→H—C(5')(+) and H—C(1')(++); H—C(1')→ *o*-H_{arom} (+) and H-C(6) (+); H—C(3')→*o*-H_{arom} ((+)) and H—C(1') ((+)); H—C(5') → *o*-H_{arom} (+) and H—C(6) ((+)); H—C(2'a)→H-C(5') (+).
^{13}C-NMR ($CDCl_3$ + 10% CD_3OD):12.48 (*C*H_3—C(5)), 40.07 (C(2')), 63.94 (C(5')), 72.36 (C(3')), 83.20 (C(1')), 98.19 (C(4')), 109.3 (C(5)), 128.1-136.2 (C_{arom}), 136.8 (C(6)), 150.4 (C(2)), 163.6 (C(4)), 195.3 (Ph*C*O).
FAB-MS: 347 (59, $[M+1]^+$). Anal. calc. for $C_{17}H_{18}N_2O_6 \cdot 0.75\ H_2O$ (359.85):
C 56.67, H 5.60, N 7.78; found: C 56.57, H 5.52, N 7.75.

Example 16: 4'-Benzoyl-2'-deoxy-5'-O-(4,4'-dimethoxytriphenylmethyl)-thymidine

Compound of formula 13 (38 mg, 0.109 mmol) is converted into 56 mg (80%) of a compound of formula 20 as described in example 6 and purified using flash chromatography (ethyl acetate/pentane/triethylamine 1:3:0.01).

IR (KBr): 3392, 3059, 2954, 2835, 1685, 1607, 1508, 1465, 1446, 1278, 1251, 1177, 1074, 1033, 829, 696.

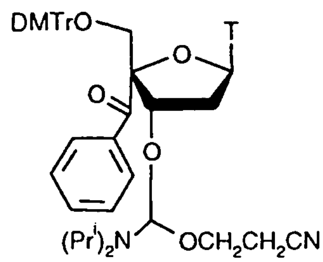


(20)

^{1}H-NMR ($CDCl_3$): 1.50 (*s*, CH_3—C(5)), 2.40 (*m*, H—C(2'b), H—C(2'a)), 3.55 (*d*, *J*=9.9, H—C(5'b)), 3.69 (*d*, *J*=9.9, H—C(5'a)), 3.78 (*s*, CH_3O), 4.75 (*dd*, *J*=1.9, *J*=3.6, H—C(3')), 6.51 (*dd*, *J*=5.7, *J*=8.8, H—C(1')), 6.79-7.45 (*m*, H_{arom}), 7.50 (*s*, H—C(6)), 7.96 (*d*, *J*=6.2, *o*-H_{arom}).
^{13}C-NMR ($CDCl_3$): 11.86 (CH_3—C(5)), 39.08 (C(2')), 55.22 (CH_3—O), 67.61 (C(5')), 75.55 (C(3')), 85.81 (C(1')), 87.95 (*C*Ar_3), 95.92 (C(4')), 111.4 (C(5)), 113.3 (C_{arom}—*ortho* to OMe), 127.2-143.8 (C_{arom}), 135.4 (C(6)), 150.2 (C(2)), 158.7 (C_{ipso}—OMe), 163.6 (C(4)), 202.8 (Ph*C*O).
FAB-MS: 648 (1.7, $[M+1]^+$). Anal. calc. for $C_{38}H_{36}N_2O_8 \cdot 0.5\ H_2O$ (657.72):
C 69.12, H 5.62, N 4.26; found: C 69.02, H 5.55, N 4.18.

Example 17: O-{3-[4-benzoyl-2-deoxy-5-O-(4,4'-dimethoxytriohenylmethyl)-1-(1-thymyl)]-ß-D-erythro-pento-1,4-furanosyl}-O-(2-cyanoethyl)-N,N'-diisopropyl-phosphoramidite

The compound of formula 20 (50 mg, 0.077 mmol) is converted into 50 mg (76%) of a compound of formula 21 as described in example 7 and purified using flash chromatography (ethyl acetate/pentane/triethylamine 2:1:0.01).



(21)

IR (KBr) mixture of diastereomers: 3177.8, 3066.7, 2966.7, 1686.3, 1605.6, 1511.1, 1466.7, 1250.0, 1177.8, 1122.2, 1072.2, 1033.3, 827.8, 755.6, 700.0.

^{1}H-NMR ($CDCl_3$), diastereomer a: 1.17 (*m*, CH(CH_3)$_2$), 1.48 (*s*, CH_3—C(5)), 2.42 (*m*, CH_2CN), 2.65 (*dd*, *J*=5.0, *J*=13.2, H—C(2b'), 2.78 (*dd*, *J*=3.7, *J*=13.2, H—C(2a'), 3.45 (*m*, CH_2—OP, CH—N, H—C(5'), CH_3O), 4.90 (*dd*, *J*=4.6, *J*=8.8, H—C(3')), 6.60 (*dd*, *J*=3.7, *J*=5.0, H—C(1')), 6.83 (*m*, H_{arom}), 7.12-7.52 (*m*, H_{arom}), 7.66 (*s*, H—C(6)), 7.82 (*d*, *J*=7.1, *o*-H_{arom}), 8.67 (s, NH), diastereomer b: 1.17 (*m*, CH(CH_3)$_2$), 1.55 (*s*, CH_3—C(5)), 2.37 (*m*, CH_2CN, H—C(2'), 3.61 (*m*, CH_2—OP, CH—N, H—C(5'), CH_3O), 5.00 (*dd*, *J*=4.1, *J*=11.9, H—C(3')), 6.68 (*dd*, *J*=2.3, *J*=7.7, H—C(1')), 6.75 (*m*, H_{arom}), 7.14-7.61 (*m*, H_{arom}), 7.62 (*s*, H—C(6)), 8.09 (*d*, *J*=7.3, *o*-H_{arom}), 8.67 (s, NH).

^{13}C-NMR ($CDCl_3$). diastereomer a: 11.70 (CH_3—C(5)), 20.19 (*d*, *J*=7.1, CH_2CN), 24.13 (*d*, *J*=7.0, N(CH(CH_3)$_2$), 24.29 (*d*, *J*=7.0, N(CH(CH_3)$_2$), 43.54 (*d*, J=12.5, N(CH(CH_3)$_2$), 47.64 (*d*, *J*=7.0, C(2')), 55.24 (CH_3—O), 58.50 (d, *J*=18.9, CH_2—OP), 67.33 (C(5')), 77.50 (*d*, *J*=18.9, C(3')), 85.83 (C(1')), 87.69 (CAr_3), 96.21 (*d*, *J*=6.1, C(4')), 112.9 (C(5)), 113.3 (C_{arom} *ortho* to OMe), 117.5 (CN), 127.1-143.9 (C_{arom}), 135.7 (C(6)), 150.2 (C(2)), 158.7 (C_{ipso}—OMe), 163.7 (C(4)), 199.5 (Ph-*C*O), diastereomer b: 12.35 (CH_3—C(5)), 19.94 (*d*, *J*=7.8, CH_2CN), 24.18 (*d*, *J*=7.2, N(CH(CH_3)$_2$), 24.39 (*d*, *J*=7.2, N(CH(CH_3)$_2$), 43.49 (*d*, J=12.9, N(CH(CH_3)$_2$), 47.41 (*d*, *J*=7.2, C(2')), 55.18 (CH_3-O), 58.35 (*d*, *J*=19.1, CH_2—OP), 67.33 (C(5')), 77.47 (*d*, *J*=19.2, C(3')), 85.65 (C(1')), 87.69 (CAr_3), 96.52 (*d*, *J*=6.3, C(4')), 111.1 (C(5)), 113.3 (C_{arom} *ortho* to OMe), 117.5 (CN), 127.1-143.9 (C_{arom}), 135.6 (C(6)), 150.1 (C(2)), 158.4 (C_{ipso}—OMe), 163.8 (C(4)), 200.1 (Ph-*C*O).

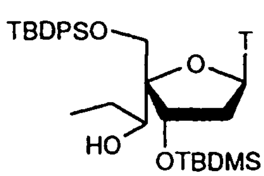
^{31}P-NMR ($CDCl_3$), diastereomer a: 149.8, diastereomer b: 149.4.

FAB-MS mixture of diastereomers: 849 (1.1, [M+1]$^+$). Anal. calc. for mixture of diastereomers $C_{47}H_{53}N_4O_9P \cdot H_2O$ (866.49):

C 65.60, H 6.39, N 6.46; found: C 65.57, H 6.42, N 6.61.

Example 18: 3'-O-tert-Butyldimethylsilyl-5'-O-tert-butyldiphenylsilyl-2'-deoxy-4'-(1-hydroxypropyl)-thymidine

To a solution of a compound of formulas (62 mg, 0.10 mmol) in THF (2 ml) a 1.5 M solution of EtMgBr (0.3 ml, 0.45 mmol) in ether is added at -78°C. After stirring for 1.5 h at - 78°C a sat. aq. NH_4Cl soln. is added (20 ml) and extracted three times with CH_2Cl_2 (50/20/20 ml). The combined organic phases are washed with water (20 ml), dried over $MgSO_4$, and evaporated *in vacuo*. Flash-chromatography (pentane : acetone 3:1) yields 41 mg (63 %) diastereomer a of formula 22 as a colorless glass. Diastereomer b is obtained as a mixture with an Isomer, where the TBDMS group has moved from the 3'-position (12 mg, 18 %, ratio (22) : Isomer= 1 : 2).



(22)

IR (KBr): 3323, 3233, 3074, 2954, 2931, 2893, 2857, 1718, 1702, 1683, 1472, 1428, 1273, 1205, 1086, 1036, 832, 778, 710, 700, 505.

^{1}H-NMR ($CDCl_3$): diastereomer a: 0.12 (*s*, CH_3—Si), 0.16 (*s*, CH_3—Si), 0.93 (*s*, *t*-Bu—Si), 0.96 (*t*, *J*=7.2, CH_3),

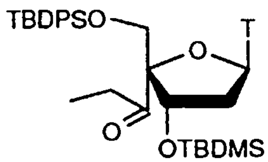
1.10 (*s*, *t*-Bu—Si), 1.20 (*m*, CH_2(a)—CHOH), 1.54 (*d*, *J*=1.2, CH_3—C(5)), 1.66 (*m*, CH_2(b)—CHOH), 2.30 (*m*, H—C(2a')), 2.38 (*ddd*, *J*=3.9, *J*=6.3, *J*=13.4, H—C(2b')), 2.80 (*dd*, ^4J=1.2, *J*=4.1, OH), 3.78 (*ddd*, *J*=2.0, *J*=4.1, *J*=10.8, *H*—COH), 3.96 (*d*, *J*=11.4, H— C(5a')), 4.10 (*d*, *J*=11.4, H—C(5b')), 4.81 (*dd*, *J*=3.9, *J*=6.3, H—C(3')), 6.31 (*dd*, *J*=6.3, *J*=7.3, H—C(1')), 7.42 (*m*, H_{arom}, H—C(6)), 7.65 (*m*, H_{arom}), 8.96 (*s*, NH). diastereomer b: 0.09 (*s*, CH_3—Si), 0.13 (*s*, CH_3—Si), 0.91 (*s*, *t*-Bu—Si), 0.94 (*t*, *J*=7.2, CH_3), 1.09 (*s*, *t*-Bu—Si), 1.40 (*m*, CH_2—CHOH), 1.57 (*s*, CH_3—C(5)), 2.30 (*m*, H—C(2')), 3.03 (*dd*, *J*=1.5, *J*=2.9, OH), 3.67 (*d*, *J*=11.0, H—C(5a')), 3.85 (*d*, *J*=11.2, H—C(5b')), 3.95 (*m*, *H*—COH), 4.75 (*dd*, *J*=2.7, *J*=6.8, H— C(3')), 6.45 (*dd*, *J*=6.1, *J*=8.1, H—C(1')), 7.40 (*m*, H_{arom}, H—C(6)), 7.64 (*m*, H_{arom}), 8.46 (*s*, NH).

^{13}C-NMR ($CDCl_3$), diastereomer a: -5.1 (CH_3—Si), -4.4 (CH_3—Si), 11.3 (CH_3—CH_2), 11.9 (CH_3—C(5)), 17.8 (Me_3C—Si), 19.4 (Me_3C—Si), 23.7 (CH_2—CHOH), 25.7 (($(CH_3)_3$—C-Si), 27.1 (($(CH_3)_3$-C-Si), 41.5 (C(2')), 63.6 (C(5')), 73.5, 73.8 (Et-*C*HOH, C(3')), 83.4 (C(1')), 89.4 (C(4')), 111.3 (C(5)), 127.9, 128.0 (m-C_{arom}), 130.0, 130.2 (p-C_{arom}), 132.3, 132.9 (i-C_{arom}), 135.1 (C(6)), 135.2, 135.4 (o-C_{arom}), 150.3 (C(2)), 163.7 (C(4)).

FAB-MS: 653 (3, $[M+1]^+$).

Example 19: 4'-Propionyl-3'-O-tert-butyldimethylsilyl-5'-O-tert-butyldiphenylsilyl-thymidine

1,1,1-Triacetoxy-1,1-dihydro-1,2-benzodioxol-3(1*H*)-one (156 mg, 2.12 mmol) and the compound of formula 22 (diastereomer a, 96 mg, 0.15 mmol) are dissolved in CH_2Cl_2 (5 ml) at 25°C. After stirring for 1 h the reaction mixture is poured in a mixture of a sat. aq. $NaHCO_3$ soln. (10 ml) and a sat. aq. $Na_2S_2O_3$ son. (10 ml), extracted three times with tert-butyl methyl ether (20 ml each), dried over $MgSO_4$, and evaporated *in vacuo*. Flash chromatography (acetone/pentane 1:3) gives 75 mg (78 %) of compound (23) as a colorless foam.



(23)

IR (KBr): 3414, 3192, 3072, 2955, 2931, 2886, 2858, 1718, 1700, 1472, 1428, 1279, 1254, 1114, 1076, 1035, 958, 834, 779, 703, 505.

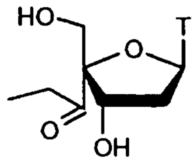
^{1}H-NMR ($CDCl_3$): -0.03 (*s*, CH_3—Si), 0.03 (*s*, CH_3—Si), 0.84 (*s*, *t*-Bu—Si), 0.96 (*t*, *J*=7.1, CH_2—CH_3), 1.10 (*s*, *t*-Bu—Si), 1.59 (*s*, CH_3—C(5)), 2.28 (*m*, H—C(2')), 2.58 (*qd*, *J*=7.1, *J*=19.4, CH_2(a)—C(O)), 2.82 (*qd*, *J*=7.1, *J*=19.4, CH_2(b)—C(O)), 3.94 (*d*, *J*=11.2, H—C(5'a)), 4.11 (*d*, *J*=11.2, H—C(5'b)), 4.49 (*d*, *J*=3.6, H—C(3')), 6.65 (*dd*, *J*=5.7, *J*=8.9, H—C(1')), 7.42 (*m*, H_{arom}), 7.63 (*m*, H—C(6), H_{arom}), 9.05 (*s*, NH).

^{13}C-NMR ($CDCl_3$): -5.3 (CH_3—Si), -5.2 (CH_3—Si), 6.6 (CH_3—CH_2), 12.0 (CH_3—C(5)), 17.9 (Me_3C—Si), 19.4 (Me_3C—Si), 25.6 (($(CH_3)_3$—C-Si), 27.0 (($(CH_3)_3$—C-Si), 33.6 (CH_2—C(O)), 41.6 (C(2')), 67.0 (C(5')), 76.0 (C(3')), 86.2 (C(1')), 96.5 (C(4')), 111.3 (C(5)), 128.0, 128.1 (m-C_{arom}), 130.1, 130.3 (p-C_{arom}), 132.0, 132.6 (i-C_{arom}), 135.26 (C(6)), 135.31, 135.5 (o-C_{arom}), 150.3 (C(2)), 163.8 (C(4)), 210.8 (Et-*C*(O)).

FAB-MS: 651 (3, $[M+1]^+$). Anal. calc. for $C_{35}H_{50}N_2O_6Si_2$ (650.97):
C 64.58, H 7.74, N 4.30; found: C 64.46, H 7.61, N 4.11.

Example 20: 4'-Propionyl-2'-deoxy-thymidine

To a solution of compound 23 (0.23 g, 0.35 mmol) in THF (15 ml) a 1M solution of tetrabutylammonium fluoride (TBAF) (0.9 ml, 0.9 mmol) is added at 25°C and stirred for 5 h. Then, silica gel (5 g) is added to the reaction mixture and the solvent is evaporated *in vacuo*. Flash chromatography (ethyl acetate/acetonitrile 4:1), removing of the solvent in vacuo, dissolving in water and lyophylization gives 72 mg (67%) of compound of formula 24 as a colorless foam.



(24)

IR (KBr): 3412, 3063, 2979, 2939, 1700, 1474, 1406, 1376, 1115, 1047, 962, 779, 572.
^{1}H-NMR (CD_3OD): 0.99 (*t*, *J*=7.2, CH_2—CH_3), 1.89 (*d*, *J*=1.2, CH_3—C(5)), 2.30 (*m*, H—C(2')), 2.68 (*m*, CH_2—C(O)), 3.84 (*d*, *J*=11.7 H—C(5'a)), 3.89 (*d*, *J*=11.7 H—C(5'b)), 4.46 (*m*, H— C(3')), 6.57 (*dd*, *J*=6.1, *J*=8.5, H—C(1')), 7.85 (*d*, *J*=1.2, H—C(6)).
^{13}C-NMR (CD_3OD): 7.3 (CH_2—*CH_3*), 12.5 (*CH_3*—C(5)), 34.8 (*CH_2*—(O)), 41.2 (C(2')), 65.6 (C(5')), 75.5 (C(3')), 87.8 (C(1')), 98.0 (C(4')), 112.0 (C(5)), 138.2 (C(6)), 152.5 (C(2)), 166.6 (C(4)), 214.3 (CH_3—*C*(O)).
FAB-MS: 299 (31, $[M+1]^+$). Anal. calc. for $C_{13}H_{18}N_2O_6 \cdot 0.35\ H_20$ (304.61):
C 51.26, H 6.19, N 9.20, O 33.35; found: C 51.13, H 6.24, N 9.05, O 33.05.

Example 21: Use of modified nucleotide in chain elongation

The triposphates of the modified nucleotides are synthesized as described by Kovács & Ötvös (Tetrahedron Lett. (1988), **29**, 4525-2528)

5' P^{32}-GTGGTGCGAATTCTGTGGAT-OH SEQ ID NO:1
3' HO-CACCACGCTTAAGACACCTA**GTCGTTCCTACTTGCTGGCT**-OH SEQ ID NP:2

A solution of template (3.0 pmol of SEQ ID NO:2) and radioactive primer (0.8 pmol of SEQ ID NO:1) in 72 µl buffer (20 mM Tris-HCl, pH 7.5; 6 mM $MgCl_2$; 40 mM KCl; 0.5 mM DTT) are heated to 80°C and allowed to cool down to 25°C within one hour. To this solution polymerase (see table 1) is added. Aliquots of thereof are mixed with 4 µl of a solution comprising dATP, dGTP, dCTP and the modified nucleotide (see table 1), resulting in a final concentration of dATP, dGTP and dCTP as provided in table 1 and a final concentration of the modified nucleotide of 100 µM. The solutions are incubated at 37°C and the elongation reaction is stopped by addition of 5 µl of a solution of formamide comprising 5 mM EDTA (pH 8.0).

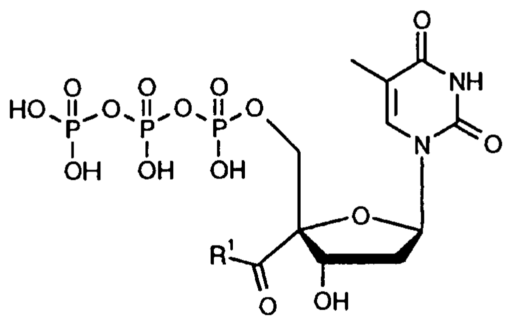
Table 1

Enzyme	Amount of enzyme	Buffer	Concentration of dATP, dGTP, dCTP
Klenow	1.5 units	10 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 7.5 mM DTT	0.5 µM each
T7 sequenase version 2	0.13 units	40 mM Tris-HCl (pH 7.5), 20 mM $MgCl_2$, 50 mM NaCl	2.5 µM each
AMV RT	2.0 units	50 mM Tris-HCl (pH 8.3), 8 mM $MgCl_2$, 30 mM KCl	0.5 µM each
M-MuLV RT	9 units	50 mM Tris-HCl (pH 8.3), 8 mM $MgCl_2$, 10 mM DTT	15.0 µM each
HIV-1RT	0.13 units	20 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 40 mM KCl, 0.5 mM DTT	1.0 µM each

3-5 µl of these solutions are subjected to a polyacrylamide gel (19 % acrylamide, 7 M urea). After electrophoresis the gel is transferred to a filter paper, dried and analyzed using autoradiography (see table 2).

Table 2

25, modified nucleotide	Klenow	T7 sequenase version 2	AMV RT	M-MuLV RT	HIV-1RT
R^1=CH_3	++	++	++	++	++ and elongation
R^1=CH_3CH_2	+	++	++	++	++
R^1=C_6H_5	-	-	++	-	++
R^1=t-butyl	-	-	-	-	-
+ = slow addition of modified nucleotide to the primer ++ = good addition of modified nucleotide to the primer - = no addition of modified nucleotide to the primer					



(25)

Example 22: Photolysis of the modified oligonucleotides

A solution of template (3.0 pmol of SEQ ID NO:2) and radioactive primer (0.8 pmol of SEQ ID NO:1) in 72 µl buffer (20 mM Tris-HCl, pH 7.5; 6 mM $MgCl_2$; 40 mM KCl; 0.5 mM DTT) are heated to 80°C and allowed to cool down to 25°C within one hour. To this solution 2.4 units of HIV-1 RT (24 µl of a 0.1 units/µl solution in 20 mM Tris-HCl (pH 7.5), 1 mM EDTA (pH 8.0), 1 mM DTT, 15 % Glycerol, 0,2 % Bovine serum albumin) is added. The solution is added to 64µl of a solution comprising dATP, dGTP, dCTP and the modified nucleotide 25 wherein R^1 is CH_3, resulting in a final concentration of dATP, dGTP and dCTP of 1.25 µl each and a final concentration of the modified nucleotide of 100 µM. After 4 h 160 µl of a 5 M NH_4OCOCH_3 solution and 1000 µl ethanol are added an cooled at -20°C for 1 h. The solution is centrifuged at 20800g, the supernatant is decanted and the residue resolved in 200 µl buffer (100 mM NaCl, 1 mM phosphate-buffer (pH 7.0), 0.01 mM EDTA).

The solution is irradiated at 15°C (Osram Hochdrucklampe, 500 W, 295 nm filter). After irradiation for 40 min. 20 µl 3M $NaOCOCH_3$ and 800 µl ethanol are added at - 20°C for 1h. The solution is centrifuged at 20800 g, the supernatant decanted and the residue resolved in 100 µl buffer (10mM Tris-HCl, pH 8.3). A 5 gl aliquot of this solution is mixed with 5 µl of a solution of formamide comprising 5 mM EDTA (pH 8.0) and loaded on a gel as described above. The autoradiogram shows the complete cleavage of the enzymatically synthesized oligonucleotides.

Sequence listing

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: CIBA-GEIGY AG
(B) STREET: Klybeckstr. 141
(C) CITY: Basel
(E) COUNTRY: Switzerland
(F) POSTAL CODE (ZIP): 4002
(G) TELEPHONE: +41 61 69 11 11
(H) TELEFAX: + 41 61 696 79 76
(I) TELEX: 962 991

(ii) TITLE OF INVENTION: Modified Nucleotides

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTGGTGCGAA TTCTGTGGAT 20

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

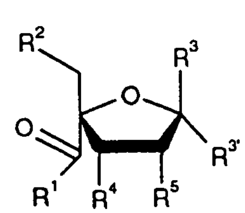
(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:complement (1..40)

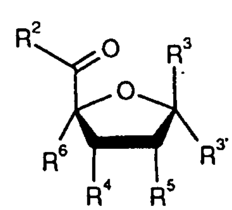
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CACCACGCTT AAGACACCTA GTCGTTCCTA CTTGCTGGCT 40

Claims

1. A compound of formula 1 or 2


(1),


(2)

wherein

R^1 is CH_3, C_2H_5, CH_2X, CHX_2, CX_3, C(O)H, C(O)C_1-C_4alkyl, CH_2OH, CH_2-O-C_1-C_4alkyl, CH_2-O-phenyl, phenyl, or phenyl substituted with nitro or X;

R^2 is OR^7, mono-, di-, or triphosphate, or esters or prodrugs thereof;
one of the residues R^3 and $R^{3'}$ is a purine or pyrimidine residue or an analogue thereof, and the other

is hydrogen;

R^4 and R^5 are independent of one another H, OR^7, $O\text{-}C_1\text{-}C_4alkylNHR^7$, $O\text{-}C_1\text{-}C_4alkylNR^7{}_2$, $-O\text{-}(CH_2\text{-}CH_2\text{-}O)_{1\text{-}4}R^7$ or $-O\text{-}CH_2\text{-}C(OR^7)H\text{-}CH_2\text{-}OR^7$;

R^6 is H, OH, CH_2OH, CH_3, CH_2CH_3, CH_2CH_2OH;

R^7 is H or an NH or OH-protecting group;

R^8 is H or an OH-protecting group;

X is F, Cl, Br or I;

or a pharmaceutically acceptable salt or prodrug thereof.

2. A compound according to claim 1, wherein R^1 is CH_3, C_2H_5, CF_3, CH_3OH, $CH_3\text{-}O\text{-}CH_3$, phenyl and phenyl substituted with nitro.

3. A compound according to claim 1, wherein R^1 is CH_3, C_2H_5 phenyl.

4. A compound according to claim 1, wherein R^1 is CH_3.

5. A compound according to claim 1, wherein R^2 is mono-, di-, or triphosphate; or HO.

6. A compound according to claim 1, wherein one of the residues R^3 and $R^{3'}$ is adenine, inosine, N-methyladenine, N-benzoyladenine, 2-methylthioadenine, 2-aminoadenine, 6-hydroxypurine, 2-amino-6-chloro purine, 2-amino-6-methylthiopurine, guanine, N-isobutyrylguanine, uracil, thymine, cytosine, 5-fluorouracil, 5-chlorouracil, 5-bromouracil, dihydrouracil and 5-methylcytosine; or their base-protected derivatives; and the other residue is hydrogen.

7. A compound according to claim 1, wherein one of the residues R^3 and $R^{3'}$ is adenine, inosine, guanine, uracil, thymine or cytosine; and the other residue is hydrogen.

8. A compound according to claim 1, wherein one of the residues R^3 and $R^{3'}$ is thymine; and the other residue is hydrogen.

9. A compound according to claim 1, wherein R^4 and R^5 are independent of one another H, or OR^7.

10. A compound according to claim 1, wherein R^4 is OH.

11. A compound according to claim 1, wherein R^5 is H.

12. A compound according to claim 1, wherein R^6 is H, OH or CH_2OH.

13. A compound according to claim 1, wherein R^7 is H or $C_1\text{-}C_4$alkyl.

14. A compound according to claim 1, wherein R^7 is H.

15. A compound according to claim 1, wherein X is F or Cl.

16. A compound according to claim 1, wherein X is F.

17. A compound according to claim 1, which has formula 1.

18. A compound according to claim 1, wherein $R^{3'}$ is hydrogen

19. Use of a compound according to claim 1 in a method of treatment.

20. Use of a compound according to claim 1 in a method of treatment, wherein the active compound according to claim 1 is formed *in vivo*.

21. Use of a compound according to claim 1 in a method of treatment of retro virus induced diseases.

22. Use of a compound according to claim 1 in a method of treating AIDS.

23. Use of a compound according to claim 1 in a method of inhibiting the proliferation of retro viruses.

24. Use of a compound according to claim 1 in a method of inhibiting the proliferation of MMTV, MLV, spumavirus, HTLV, BLV, lentivirus, HIV, HSV and SIV.

25. Use of a compound according to claim 1 in a method of inhibiting HIV.

26. Pharmaceutical composition comprising a compound according to claim 1 optionally together with pharmaceutical acceptable carrier.

27. Method for the preparation of a pharmaceutical composition according to claim 26.

28. Use of the compound according to claim 1 for the inhibition of reverse transcriptase.

29. Use of a compound according to claim 1 in a screen.

30. Use of a compound according to claim 29 in a screen for the identification of the presence or absence of reverse transcriptase.

31. Use of a compound according to claim 29 in a screen for the identification of HIV-1RT.

32. Use of a compound according to claim 1 for the generation of photolytically cleavable DNA and RNA and analogues thereof.

33. A photocleavable linker comprising a compound according to claim 1.

34. Method for the synthesis of a compound according to claim 1.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 96 81 0216

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	EP 0 222 330 A (WARNER-LAMBERT COMPANY) 20 May 1987 * preparations 1-3; examples 1-9 *	1,5-7,9, 10	C07H19/04 A61K31/70
X	DE 22 44 215 A (ABBOTT LABORATORIES) 15 March 1973 * claims 1,10,11; examples 1B,1D,3,5-8 *	1,6,7,9, 10	
X	EP 0 515 156 A (BIOCHEM PHARMA INC.) 25 November 1992 * scheme 4, compound XII; scheme 5, compound XIV; examples 4,6,7 *	1	
X	CARBOHYDRATE RESEARCH, vol. 100, 1982, AMSTERDAM NL, pages 315-329, XP002011882 C.M.RICHARDS ET AL.: "Synthesis of 4'-Methoxyadenosine and Related Compounds" * page 319, compound 15 *	1,6,7,9, 10	
X	JOURNAL FÜR PRAKTISCHE CHEMIE CHEMIKER-ZEITUNG, vol. 335, 1993, pages 415-424, XP002011883 E.DIEKMANN ET AL.: "Didesoxy-Ribonucleoside durch Schmelzkondensation." * page 415, compounds 6a-d *	1,9,10	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C07H A61K
X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 17, no. 7, July 1974, WASHINGTON US, pages 764-766, XP002011884 J.J.BAKER ET AL.: "Thymidine 5' Variants as Inhibitors of Thymidylate Kinase" * page 765, chart I, compounds 2,3a,3b,3c*	1,6-10	

-/--

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	7 February 1997	Scott, J

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 96 81 0216

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	TETRAHEDRON LETTERS, vol. 33, no. 1, 1992, OXFORD GB, pages 41-44, XP002011885 C.O-YANG ET AL.: "4'-Substituted Nucleosides as Inhibitors of HIV : An Unusual Oxetane Derivative." * the whole document * ---	1	
A	US 5 192 749 A (O-YANG ET AL.) 9 March 1993 * the whole document * -----	1	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	7 February 1997	Scott, J

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
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L : document cited for other reasons

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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filling more than ten claims

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions, namely:

See sheet -B-

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid,

namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims,

namely claims: 1-10

European Patent Office

EP 96 81 0216 - B -

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions, or groups of inventions, namely:

1. Claims 1,6-10, in particular 2-5, wholly: Compounds of formula (1), claim 1, their preparation, pharmaceutical composition containing them and their use.
2. Claims 1,6-10 : Compounds of formula (2) claim 1, their preparation, pharmaceutical composition containing them and their use as antiviral agents.
3. Claims 1,6-10 : Compounds of formula (2), claim 1, their preparation, pharmaceutical composition containing them and their use as a screening agent.
4. Claims 1,6-10 : Compounds of formula (2) claim 1, their preparation, pharmaceutical composition containing them and their use as a photocleavage agent.

EPO Form Supplementary Sheet B (1996)